3180-Pos Board B285
New Obscurins Play a Role in Cardiac Electrochemical Signaling
Maegen A. Ackermann, Jane Valenti,
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Obscurins are a family of modular proteins expressed in striated muscle cells. Alternative splicing events give rise to various isoforms containing a combination of adhesion modules and signaling motifs. The prototypical obscurin (obscurin-A) is a giant (~720 kDa) multidomain protein that intimately surrounds sarcomeres at the level of M-bands and Z-disks where it is appropriately positioned to participate in their assembly, stability and integration with other sarcoplasmic elements.
Our laboratory has recently identified a novel isoform of obscurin (referred to as obscurin-D), that results from complex differential shuffling at the 3' end of the OBSCN gene leading to the usage of an alternate initiation codon within exon 82. The obscurin-D transcript encodes a protein that contains tandem rho-guanine nucleotide exchange factor (Rho-GEF) and pleckstrin homology (PH) motifs followed by two immunoglobulin (Ig) domains and non-modular sequences. Using immunofluorescence combined with confocal microscopy and Z-stack sectioning, we found that obscurin-D localizes at the intercalated disc (ICD), the unique membrane microdomain of cardiomyocytes that contributes to the electrical and mechanical coupling of neighboring cells. This finding was further confirmed by immunoelectron microscopy, which indicated the presence of obscurin-D throughout the length of the ICD, at the transitional zone. Consistent with this, biochemical assays demonstrated that obscurin-D is in a complex with major ICD proteins, including N-cadherin, vinculin and connexin-43, and may bind to membranes through its PH domain, following activation of phosphoinositide 3 kinase (PI3K). Experiments are currently under way to examine the roles of obscurin-D at the ICD by manipulating its expression levels through adeno-associated mediated viral gene delivery in whole animals.

3181-Pos Board B286
How to Catch Moby Dick: Systematic Identification of Binding Partners for UNC-89 (Obscurin)
Hiroshi Qadota, Ge Xiong, Kristy Wilson, M. Berenice Duran,
Emily Swartzbaugh, John Nahabedian, **Guy Benian**.
Mutations in *unc-89* result in adult *C. elegans* with reduced motility and disorganized, thinner sarcomeres. UNC-89-B (8,081 residues) has 53 Ig domains, 2 Fn3 domains, SH3, DH and PH domains at its N-terminus, and 2 protein kinase domains (PK1 and PK2) at its C-terminus. The human homolog is called "obscurin". Antibodies localize UNC-89 to the M-line. To understand how UNC-89 is localized and how it performs its functions, we are systematically identifying its binding partners. UNC-89-B is entirely represented as 16 overlapping segments in 2-hybrid vectors. All 16 have been used to screen ~23 known components of the nematode M-line. 7/16 have been used to screen a 2-hybrid library. We have identified 6 partners, each with human homologs, and all but one localized to M-lines. Both PK1 and PK2 interact with SCPL-1, a CTD-type protein phosphatase. *scpl-1* mutants display defective egg laying muscles, and "hyper-bending" during locomotion. PK1 and the "interkinase region" interact with LIM-9 (FHL). *lim-9(RNAi)* show aggregates of myosin. Ig50-Ig51 interacts with HIF-1 (hypoxia inducible factor), and Fn1-Ig52 interacts with HUM-6 (class VII myosin). Ig1-Ig2-Ig3 interacts with CPNA-1, a copine domain containing protein. Loss of function for *cpna-1* results in a Pat embryonic lethal phenotype, characteristic of mutations in 19 other genes, most of which encode products associated with integrin associated muscle adhesions. Three segments, Ig9-Ig11, Ig18-Ig23, and Ig50-Ig51 interact with CUL-1, a cullin, known to act as a scaffold for assembly of the ubiquitylation protein degradation machinery. *cul-1(RNAi)* shows disorganization of thick filaments in a pattern similar to that of *unc-89(su75)*, an allele lacking all CUL-1 binding sites. My talk will focus on the role of CPNA-1 in localizing UNC-89, and the UNC-89/CUL-1 interaction as a novel means of regulating protein degradation in muscle.

3182-Pos Board B287
Interaction of Obscurin a with Small Ankyrin 1
Robert J. Bloch, Ben Busby, Taiji Oashi, Chris Willis, Maegen Ackermann,
Aikaterini Kontrogianni-Konstantopoulos, Alexander D. Mackerell Jr.
We have studied the binding of a small product of the ankyrin 1 gene (sAnk1; Ank1.5), a ~17.5 kDa integral protein of the sarcoplasmic reticulum, to the C-terminus of obscurin A, a ~720 kDa protein that envelopes sarcomeres at Z-disks and M-bands. Alanine scanning mutagenesis identified several lysines and arginines in the cytoplasmic sequence of sAnk1 that mediate binding to obscurin, and several glutamates in the high affinity site of obscurin that mediate binding to sAnk1. Complementary K- or R-to-E and E-to-K mutations identified specific pairs of residues involved in binding; mutagenesis of several of these eliminates binding. Molecular and Brownian dynamics simulation suggested several possible models for the docked complex but predicted only one in which D111 of sAnk1 and K6338 of obscurin interact. We confirmed their interaction by complementary mutagenesis. We tested the model further by using similar approaches to examine the hydrophobic residues involved in binding, with results consistent with the predictions of a representative structure of the docked complex selected from cluster analysis of structures generated from molecular dynamics simulations. Our studies indicate that the obscurin-binding region of sAnk1 is comprised of two ankyrin-like repeats, which establish specific electrostatic and hydrophobic contacts with the high affinity site on obscurin, composed of an 18-residue α-helical polypeptide. We identify structures similar to this polypeptide in the binding regions of proteins that interact with other ankyrin repeat proteins. We propose that the 18-mer in the high affinity binding site on obscurin for sAnk1 represents a prototypical ankyrin binding motif.
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Novel Insights in the Function of the Giant Sarcomeric Proteins Titin and Nebulin
Henk Granzier.
The striated muscle sarcomere contains two giant proteins: titin and nebulin. Titin is a giant elastic protein (3-4 MDa) that spans the half sarcomere from Z-disc to M-band, and is responsible for a large fraction of passive stress that develops when muscle is stretched. Alternative splicing results in large fetal isoforms and smaller adult isoforms that differ mainly in the size of their extensible I-band region. Differences in the mechanical properties of these isoforms and how these properties are altered by excision of specific spring elements have been recently established using novel knockout models. Findings show that titin mechanics can be tuned by phosphorylation, including the recently discovered PKG and PKC phosphorylation pathways. Furthermore, titin-binding proteins that interact with titin along the full length of the sarcomere play a role in protein turnover, and in sensing mechanical stresses that initiate hypertrophic signaling. Nebulin is a giant 600-900 kDa filamentous protein that is an integral component of the skeletal muscle thin filament. Recent improvements in the field, especially the development of mouse models deficient in nebulin (NEB-KO mice), indicate that nebulin functions, in addition to its role in thin filament length specification, in the regulation of muscle contraction. Muscle fibers deficient in nebulin have a higher tension cost, and develop less force due to reduced myofilament calcium sensitivity and altered crossbridge cycling kinetics. These novel functions indicate that nebulin might have evolved in vertebrate skeletal muscles to efficiently develop high levels of muscle force. The NEB-KO mouse models also highlight nebulin's role in the assembly and alignment of the Z-disks. Importantly, rapid progress in understanding nebulin's in vivo roles provides clinically important insights in how nebulin deficiency in patients with nemaline myopathy contributes to debilitating muscle weakness.

3184-Pos Board B289
Deciphering the Functional Properties of Nebulin: It is a Stabilizer!
Chris T. Pappas, Paul A. Krieg, **Carol Gregorio**.
Striated muscle cells display one of the most extreme examples of molecular organization found in nature. Efficient muscle contraction requires precise regulation of actin (thin) filament lengths. In one highly cited model, the giant protein nebulin (~750-900 kDa) has been proposed to function as a "molecular ruler" specifying filament lengths. We directly challenged this hypothesis by constructing a unique, small version of nebulin (mini-nebulin). When endogenous nebulin was replaced with mini-nebulin in skeletal myocytes, thin filaments extended beyond the end of mini-nebulin; an observation that is inconsistent with a strict ruler function. However, under conditions that promote actin filament depolymerization, filaments associated with mini-nebulin were remarkably maintained at lengths either matching or longer than mini-nebulin. This indicates that mini-nebulin is able to stabilize portions of the filament it has no contact with. Knockdown of nebulin also resulted in more dynamic populations of thin filament components, while expression of mini-nebulin decreased the dynamics at both ends of the filament (i.e., efficiently recovered loss of endogenous full-length nebulin). Taken together, our data reveals that nebulin regulates thin filament architecture by a mechanism that includes stabilizing the filaments and preventing actin depolymerization.